IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application	: Michael Valentine Agrez et al.
Application No.	: 10/019,816
Filed	: March 27, 2002
Confirmation No.	: 9944
For	: METHOD OF MODULATING INTEGRIN MEDIATED CELLULAR ACTIVITY AND AGENTS USEFUL FOR SAME
Examiner	: Karen A. Canella
Attorney's Docket	: ADAM-046XX

TC Art Unit: 1643

* *

DECLARATION OF MICHAEL VALENTINE AGREZ, Ph.D.
UNDER 37 C.F.R. §1.132

Via Electronic Filing
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Michael Valentine Agrez, of 46 Sherburn Place, Charlestown, New South Wales 2300, Australia, hereby declare as follows:

1. I am an Associate Professor of The University of Newcastle, New South Wales, Australia.

2. The major thrust of my research over the last 2 decades has been directed at growth signaling in colon cancer cells. After graduating from medicine and then specializing in colorectal surgery (MB.BS, FRCS, FRACS) my two subsequent doctoral theses were in the fields of cancer cell biology (MS) and molecular cell biology (PhD). In recognition of my research achievements as a scientist in parallel with a clinical career, I was awarded the John Mitchell Crouch Fellowship in the year 2000 – the highest award offered by the Royal

WEINGARTEN, SCHURGIN,
GAGNEBIN & LEBOVICI LLP
TEL. (617) 542-2290
FAX. (617) 451-0313

Australasian College of Sugeons to one individual in Australia each year. I have authored over 30 scientific articles dealing specifically with biochemical, molecular or cellular aspects of cancer, which have been published in high ranking international peer-reviewed journals such as Journal of Cell Biology, Journal of Biological Chemistry, Oncogene, International Journal of Cancer, British Journal of Cancer, European Journal of Cancer, Journal of Virology, Molecular Biology of the Cell, Journal of Surgical Oncology and Biochemical Biophysical Research Communications amongst others.

3. I am a co-inventor of the subject matter described and claimed in the above-identified patent application.

4. I have read and understood the Office Actions of the Examiner, including the Office Action mailed June 9, 2010.

5. In the Office Action of June 9, 2010, the Examiner acknowledged the instant specification teaches that SEQ ID No. 2 (RSKAKWQTGTNPLYR), SEQ ID No. 22 (RARAKWDTANNPLYK) and SEQ ID No. 23 (RSRARYEMASNPLYR) bind to the MAP kinase Erk2. As explained in the instant application, e.g., at pp. 86 and 92, SEQ ID No. 2 was isolated from the cytoplasmic tail of the β6 integrin subunit and provides a binding domain of the β6 integrin subunit for Erk2. Likewise, SEQ ID No. 22 was isolated from the cytoplasmic tail of the β3 integrin subunit and provides a binding domain of the β3 integrin subunit for Erk2, and SEQ ID No. 23 was isolated from the cytoplasmic tail of the β5 integrin subunit and provides a binding domain of the β5 integrin subunit for Erk2.

6. The Examiner also acknowledged her understanding that SEQ ID No. 3 (RSKAKNPLYR) is derived from the β6 integrin subunit and represents SEQ ID No. 2 with a deletion of the linker amino acids WQTGT. The Examiner commented, however, that although the

WEINGARTEN, SCHURGIN,
GAGNEBIN & LEBOVICI LLP
TEL. (617) 542-2290
FAX. (617) 451-0313

specification stated that evidence of binding of Erk2 to the β3 and β5 integrin binding domain peptides was found, the specification failed to state if the corresponding amino and carboxy terminal regions in those β3 and β5 peptides would bind to Erk2 *out of context* of their positions in their respective binding domain when the intervening linker amino acids in those binding domains were deleted.

7. In a telephone conversation with the Applicants' attorney on July 30, 2010, the Examiner suggested that declaration evidence, showing that the β3 and β5 peptides without the linker sequences perform as the Applicants had asserted, would advance the prosecution of the instant application.

8. I report herein that experiments I have conducted or which have been conducted under my direction, show that the β3 derived peptide RARAKNPLYK represented by the SEQ ID No. 22 peptide with a deletion of the linker amino acids WDTAN, and the β5 derived peptide RSRARNPLYR represented by the SEQ ID No. 23 peptide with a deletion of the linker amino acids YEMAS, both inhibit the growth of cancer cells.

9. In particular, Graph A in Annexure MVA-1 attached to this declaration shows that the polypeptide AAVALLPAVLLALLARARAKNPLYK (IK3), comprising the β3 derived peptide RARAKNPLYK coupled to the partial signal peptide AAVALLPAVLLALLA and administered via subcutaneous injection to Balb/c nude mice daily over a period of 5 days at a dosage of 12 mg/kg body weight in physiological saline, inhibited the growth of DU145 human prostate cancer cell xenographs compared to normal saline alone. The partial signal peptide AAVALLPAVLLALLA was used as a "facilitator moiety" to facilitate passage of the RARAKNPLYK peptide across the outer cell membrane into the cytoplasm of the cancer cells.

WEINGARTEN, SCHURGIN,
GAGNEBIN & LEBOVICI LLP
TEL. (617) 542-2290
FAX. (617) 451-0313

10. Further, Graph B in Annexure MVA-1 shows that SEQ ID No. 23 (RSRARYEMASNPLYR) and the β5 derived peptide RSRARNPLYR coupled to the partial signal peptide AAVALLPAVLLALLA (respectively designated nf-b5-frag5 and nf-b5-10-4 in Graph B), each inhibit the proliferation of HT29 human colon carcinoma cells in an MTT cell proliferation assay. To prepare the MTT solution for use in the assay, 100 mg of MTT (methylthiazoletetrazolium) was mixed with 20 ml of phosphate buffered saline (PBS) at pH 7.4. The resulting solution was filter sterilized (0.2 μM syringe filter), stored at 4°C and protected from light until use. MTT substrate is cleaved in growing cells to yield a water insoluble salt. After solubilisation of the salt, a coloured product is produced that allows quantitation of the proliferative activity of the cells. As also shown in Graph B, while both of the administered peptide agents inhibited proliferation of the cancer cells, a greater degree of inhibition was observed for the peptide agent comprising RSRARNPLYR.

11. These results show that like SEQ ID No. 3 (RSKAKNPLYR), each of the RARAKNPLYK and RSRARNPLYR peptides inhibit the growth of cancer cells.

12. Simple sequence alignment shows 80% amino acid sequence identity between SEQ ID No. 3 (RSKAKNPLYR) and the RARAKNPLYK peptide, and also between peptides RARAKNPLYK and RSRARNPLYR. Further, a sequence identity of 70% exists between SEQ ID No. 3 (RSKAKNPLYR) and peptide RSRARNPLYR. In addition, the Examiner will note the sequence identity between the 5 C-terminal amino acids of these three peptides is 80% to 100%.

13. The high level sequence identity that exists between SEQ ID No. 3 (RSKAKNPLYR) and the RARAKNPLYK and RSRARNPLYR peptides does not exist between the deleted linker sequences WQTGT, WDTAN and YEMAS.

WEINGARTEN, SCHURGIN,
GAGNEBIN & LEBOVICI LLP
TEL. (617) 542-2290
FAX. (617) 451-0313

14. Thus, it is clear that the linker amino acids of SEQ ID No. 2 (RSKAKWQTGTNPLYR), SEQ ID No. 22 (RARAKWDTANNPLYK) and SEQ ID No. 23 (RSRARYEMASNPLYR) are not conserved, and like amino acids WQTGT in SEQ ID No. 2, the WDTAN and YEMAS linker amino acids in SEQ ID No. 22 and SEQ ID No. 23 peptides are also not important to, or required for, inhibition of cancer cell growth.

15. That SEQ ID No. 3 (RSKAKNPLYR) and the RARAKNPLYK and RSRARNPLYR peptides retain cancer cell growth inhibitory activity may be explained by the β6, β3 and β5 binding domains for Erk2 each forming a respective "loop" comprising the linker amino acids of the binding domain, thereby drawing the opposite end regions of the binding domain linearly together.

I hereby declare that all statements made herein on personal knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed this 15th day of October 2010.

By _____
Michael Valentine Agrez, Ph.D.

HCH/ker

395661.1

WEINGARTEN, SCHURGIN,
GAGNEBIN & LEBOVICI LLP
TEL. (617) 542-2290
FAX. (617) 451-0313

THIS IS ANNEXURE MVA-1

to

THE DECLARATION OF

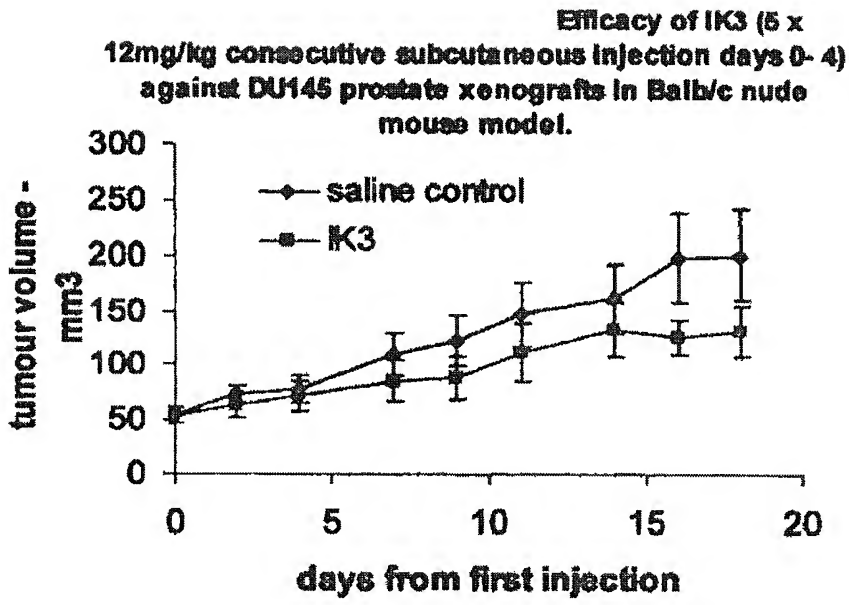
MICHAEL VALENTINE AGREZ

Signed this 15th day of October 2010.

By _____

Michael Valentine Agrez, Ph.D.

GRAPH A



GRAPH B